but 3 out of 4 DOT1L inhibited samples showed a significantly reduced effect of TGFβ on ACTA2 expression. The amount of collagen I and III in the extracellular matrix after 72 hours of TGFβ was comparable between control and EPZ treated fibroblasts. BrdU labelling assay showed increased proliferation with DOT1L inhibition. In vivo, subcutaneous bleomycin induced an increased dermal thickness and skin collagen content in mice. No difference in the effect of bleomycin was found between mice with a conditional fibroblast-specific DOT1L knockout or wild type mice.

**Conclusions** In an in vitro model of fibrosis, primary human dermal fibroblasts treated with a DOT1L-inhibitor showed increased proliferation and reduced upregulation of ACTA2 but did not result in detectable differences in collagen deposition. In an in vivo murine model of skin fibrosis, no difference in bleomycin-induced skin thickness and collagen content was found when DOT1L was knocked out in fibroblasts.

**Disclosure of interest** None declared.

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**P102 S100A9 HAMPERNS OSTEOCLAST DIFFERENTIATION FROM CIRCULATING PRECURSORS BY REDUCING THE EXPRESSION OF RANK**

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**Introduction** The alarmins S100A8/A9 are produced in high levels in the synovium during both experimental and human rheumatoid arthritis (RA) and have been implicated in inflammation-induced bone resorption. We and others have previously shown that stimulation of mature osteoclasts with S100A8/A9 results in increased numbers and resorptive activity. In agreement, reduced bone destruction was observed after induction of experimental RA models in S100a9−/− mice. However, the effects of S100A8/A9 on monocyte-to-osteoclast differentiation remain elusive.

**Objectives** Here, we investigated the effects of S100A9 on CD14+ monocytes and their potential to differentiate into osteoclasts.

**Methods** CD14+ monocytes were isolated from buffy coats of healthy donors using density gradient centrifugation and magnetic cell sorting. Cells were differentiated into osteoclasts with macrophage colony-stimulating factor (M-CSF) and Receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL) in the presence or absence of S100A9. mRNA expression was determined by RT-qPCR and protein expression was determined using Luminex analysis. Moreover, osteoclast differentiation was assessed using Tartrate-resistant acid phosphatase (TRAP) staining and the resorptive capacity was determined using mineral-coated plates. RANK protein expression was assessed using FACS.

**Results** We observed that S100A9 stimulation of monocytes resulted in a strong induction of various pro-inflammatory factors, such as interleukin (IL)1β, IL6, IL8, and tumour necrosis factor (TNFα) after 24 hour, both on the mRNA and protein level. Interestingly, we observed a strong decrease in the number of multinucleated osteoclasts as determined by TRAP staining, at day 6 and 8 after start of the cultures. In agreement with this, the cells showed a strongly reduced resorptive capacity. We demonstrated that already a 24 hour stimulation with S100A9 strongly reduced the osteoclastogenic potential of the CD14+ monocytes. Finally, we observed that S100A9 stimulation hampered the M-CSF-induced upregulation of RANK, which could be reversed by addition of the TNFα-inhibitor etanercept, but not the interleukin 1 receptor antagonist.

**Conclusions** Whereas S100A8/A9 have been previously shown to stimulate the numbers and resorptive capacity of mature osteoclasts, we here show that stimulation of monocytes with S100A9 strongly inhibits their osteoclastogenic potential, possibly via TNFα-induced reduction of RANK expression. This suggests that the timing of exposure to S100A8/A9 is an important determinant for monocyte-to-osteoclast differentiation.

**Disclosure of interest** None declared.